Transforming Growth Factor Beta Switch in Aqueous Humor of Patients With Fuchs' Endothelial Corneal Dystrophy

We have read with great interest the article by Matthaei et al.[1] on the expression of epithelial–mesenchymal transition (EMT)-related cytokines (monocyte chemoattractant protein [MCP]-1/chemokine [CC motif] ligand [CCL]2, transforming growth factor [TGF]-β1, TGF-β2, TGF-β3, basic fibroblast growth factor, tumor necrosis factor [TNF]-α, and interleukin [IL]-1β) in the aqueous humor (AH) of phakic and pseudophakic Fuchs' endothelial corneal dystrophy (FECD) eyes. With this letter, we want to confirm their findings on MCP-1, but contradict their findings on TGF-β2. Additionally, we present new data on inflammatory cytokines in the AH of FECD eyes.

Matthaei et al.[1] found a significantly elevated concentration of MCP-1, TGF-β1, and TGF-β2 in pseudophakic FECD eyes ($FECD_{psph}$) compared with cataract controls (which we will abbreviate as $Control_{ph}$) and phakic FECD eyes ($FECD_{ph}$), but they observed no differences between $FECD_{ph}$ and $Control_{ph}$. They rightly concluded that there was no primary role for these aqueous cytokines in FECD pathogenesis, but that the upregulation of these EMT-associated proteins in $FECD_{psph}$ was probably secondary to cataract surgery.

Within the frame of a study into the molecular pathogenesis of FECD, we have recently conducted a similar experiment. Unlike Matthaei et al.,[1] we also included a group with pseudophakic non-FECD edematous corneas ($Edema_{psph}$), and our analysis involved nine molecules in AH: five of them related to EMT (MCP-1, TGF-β1, TGF-β2, TGF-β3, and TNF-α), and four related to inflammation (IL-8/chemokine [CXC motif] ligand [CXCL]8, IL-6, growth-regulated oncogene α [GROα]/CXCL1 and IFN-α2). We compared $Control_{ph}$ ($n = 21$) with $FECD_{ph}$ ($n = 5$), $FECD_{psph}$ ($n = 19$), and $Edema_{psph}$ ($n = 8$). The group $Edema_{psph}$ consisted of both graft failures ($n = 2$) and pseudophakic bullous keratopathy ($n = 6$). Female/male ratio was 3:2 and all patients were older than 50 years. Magnetic bead ELISAs were purchased from Merck Millipore (Darmstadt, Germany). Plate readings were done with a suspension array system and software (Bio-Plex 200 System and Bio-Plex Manager, version 4.1.1.456; Bio-Rad Laboratories, Inc., Hercules, CA, USA). Undiluted AH was used in duplicate.

Similar to Matthaei et al.,[1] we observed a significant upregulation of MCP-1 in $FECD_{psph}$ compared with $FECD_{ph}$ ($P < 0.05$) and $Control_{ph}$ ($P < 0.001$; Fig.). In addition, we found MCP-1 to be significantly upregulated in $Edema_{psph}$ compared with $FECD_{ph}$ ($P < 0.001$) and $Control_{ph}$ ($P < 0.001$). Monocyte chemoattractant protein 1 did not differ significantly between both phakic groups and between both pseudophakic groups, respectively. This strengthens the conclusion of Matthaei et al.[1] that MCP-1 does not play a primary role in the pathogenesis of FECD, but rather that it reflects a common response after cataract surgery. Like MCP-1, IL-8 was significantly upregulated in $Edema_{psph}$ compared with both phakic groups: $Control_{ph}$ ($P < 0.001$) and $FECD_{ph}$ ($P < 0.01$). Interleukin 8 was also significantly upregulated in $FECD_{psph}$ compared with $Control_{ph}$ ($P < 0.05$). This suggests again that IL-8 does not play a primary role in the pathogenesis of FECD, but that the rise in IL-8 reflects a common response to cataract surgery. However, unlike Matthaei et al.,[1] we did not observe an upregulation of TGF-β2 in $FECD_{psph}$ compared with both phakic groups. Instead, we saw significantly less TGF-β2 in the AH of pseudophakic group "$Edema_{psph}$" compared with both phakic groups: $Control_{ph}$ ($P < 0.05$) and $FECD_{ph}$ ($P < 0.001$). In addition, there was a significant difference in TGF-β2 concentration between both phakic groups ($P < 0.05$) and between both pseudophakic groups ($P < 0.05$), respectively, with a higher concentration in patients with FECD. This suggests that TGF-β2 is in fact specific for FECD within each category of phakic or pseudophakic eyes.

Transforming growth factor β3 was below and TGF-β1 was above the detection limit in the study of Matthaei et al.[1] In our study, it was the reverse. Therefore, we cannot comment on the findings for TGF-β1, but we can give new information on TGF-β3. There was significantly more TGF-β3 in $FECD_{ph}$ compared with all other groups: $Control_{ph}$ ($P < 0.01$), $FECD_{psph}$ ($P < 0.01$), and $Edema_{psph}$ ($P < 0.001$).

Both GROα and IL-6 were specifically and significantly upregulated in $Edema_{psph}$. Interleukin 6 was upregulated in $Edema_{psph}$ compared with $FECD_{ph}$ and $FECD_{psph}$ ($P < 0.05$ for both comparisons). But there was no significant difference between $Edema_{psph}$ and $Control_{ph}$, nor between $FECD_{ph}$ or $FECD_{psph}$ and $Control_{ph}$. Concentrations of GROα did not differ between $FECD_{ph}$ or $FECD_{psph}$ and $Control_{ph}$ either, but GROα was significantly upregulated in $Edema_{psph}$ compared with all other groups ($P < 0.001$ for all comparisons). This suggests that the rise in GROα is specific for $Edema_{psph}$.

These findings are in line with microarray expression analysis (MEA) and quantitative (q)PCR data that we have produced on $FECD_{psph}$ corneal endothelium (CEn) compared with normal donor CEn (De Roo AK, Janssens T, Wouters J, Govaere O, Foets B, van den Oord JJ, unpublished observations, 2016). Quantitative PCR showed a significant upregulation of TGF-β2 and TGF-β3 and a significant downregulation of GROα in $FECD_{psph}$. Interleukin 6 and 8 were significantly downregulated in $FECD_{psph}$ based on MEA, but not significantly changed on qPCR. Despite the increased concentration of MCP-1 in the AH of $FECD_{psph}$, the corresponding mRNA was significantly downregulated according to MEA, but not significantly changed according to qPCR.

Our data on TGF-β2 and TGF-β3 combined with the data on TGF-β1 from Matthaei et al.,[1] suggest that TGF-β2 and TGF-β3 are specific for FECD and that there is a switch toward TGF-β1 after cataract surgery. Notably, all three isoforms of TGF-β (1, 2, and 3) are expressed in the normal cornea, with TGF-β2 being present at the highest concentration and TGF-β3 at the lowest concentration.[2] Interestingly, TGF-β2 is involved in tissue development[2] and TGF-β3 is known to have an antiscarring effect (counteracting TGF-β1 signaling).[3] On the other hand, TGF-β1 plays a role in wound healing and classical EMT, and has been proposed as a key player in the disease mechanism of pseudophakic bullous keratopathy.[2] Furthermore, our data suggest MCP-1 and IL-8 to rise in response to cataract surgery.

In conclusion, the TGF-β family of cytokines likely plays a role in FECD, but additional studies are required to define which members are involved in the pathogenesis, and which arise in response to surgery.

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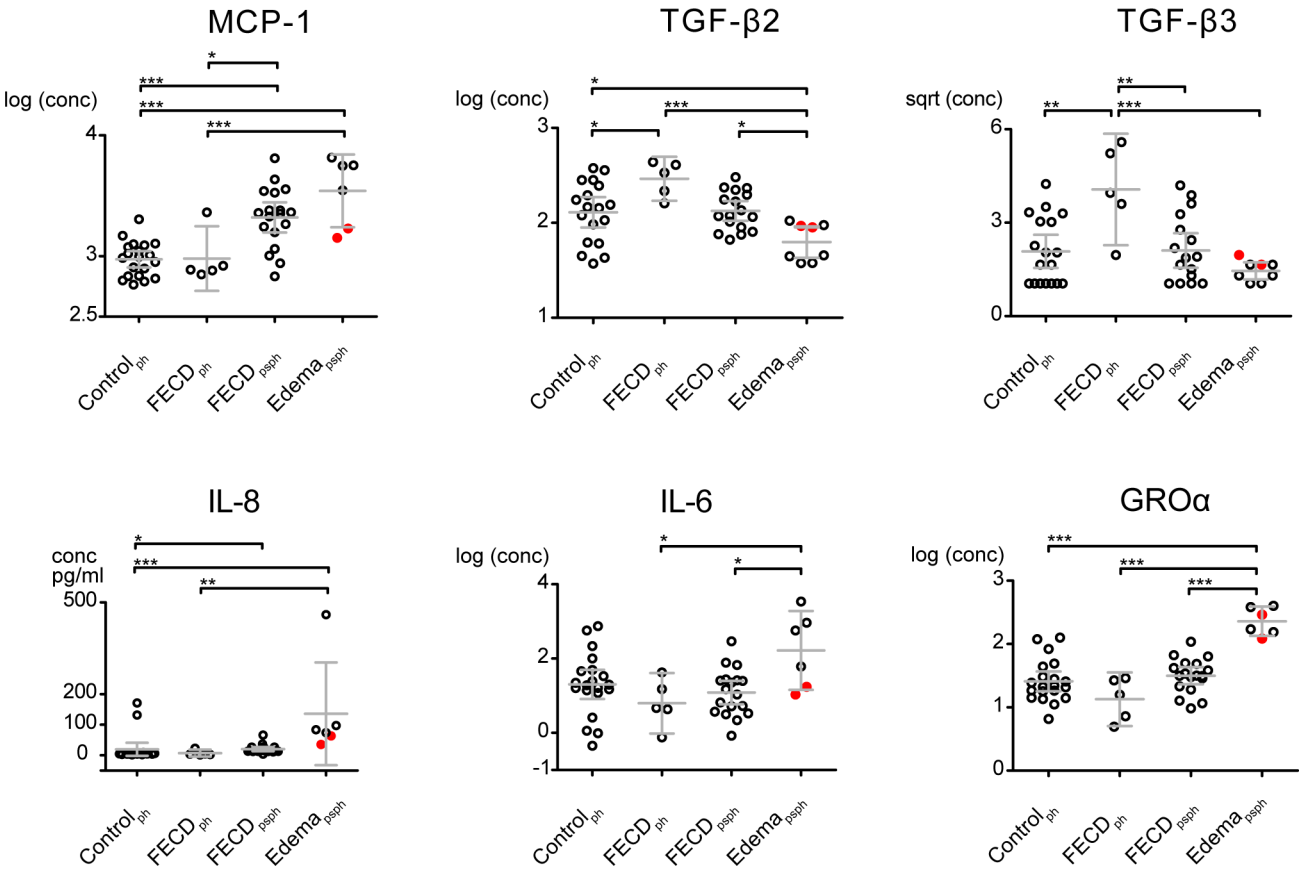

Figure. Cytokine concentrations in aqueous humor. Transforming growth factors β2 and β3 are specifically and significantly upregulated in $FECD_{ph}$. Monocyte chemoattractant protein 1 and IL-8 are significantly upregulated after cataract surgery. Interleukin 6 and GROα are specifically and significantly upregulated in $Edema_{psph}$ compared with $FECD_{ph}$ and $FECD_{psph}$. *Asterisks* in individual graphs indicate the *P* value: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. *Error bars*: display the mean and 95% confidence interval. Edema indicates non–FECD-related corneal edema and consists of both pseudophakic bullous keratopathy and graft failures. Graft failures ($n = 2$) are indicated in *red*. Distinction was made between phakic and pseudophakic eyes. Logarithmic (log) and square root (sqrt) transformations were used. The concentration itself was in pg/mL.

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